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Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Glyphosate, Aminomethylphosphonic Acid, and Glufosinate in Water Using Online Solid-Phase Extraction and High-Performance Liquid Chromatography/Mass Spectrometry

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CONVERSION FACTORS, MISCELLANEOUS ABBREVIATIONS, AND ABBREVIATED WATER-QUALITY UNITS

Conversion Factors

Multiply	By	To obtain
cubic centimeter (cm^3)	0.06102	cubic inch
gram (g)	2.205×10^{-3}	pound
liter (L)	2.642×10^{-1}	gallon
meter (m)	3.281	foot
microliter (µL)	2.642×10^{-7}	gallon
micrometer (µm)	3.937×10^{-5}	inch
milligram (mg)	3.53×10^{-5}	ounce
millimeter (mm)	3.937×10^{-2}	inch
ounce (oz)	0.02957	liter
pound per square inch (lb/in^2)	6.895	kilopascal

Temperature can be converted to degrees Celsius (°C) or degrees Fahrenheit (°F) by the equations:

$$^{o}C = 5/9\ (^{o}F - 32)$$
$$^{o}F = 9/5\ (^{o}C) + 32.$$

Miscellaneous Abbreviations

mass to charge (m/z)
minute (min)
second (sec)
volt (V)

Abbreviated Water-Quality Units

liter per minute (L/min)
microgram per liter (µg/L)
microgram per milliliter (µg/L)
milligram per milliliter (mg/mL)
milliliter (mL)
milliliter per minute (mL/min)
millimoles (mM)

Methods of Analysis by the U.S. Geological Survey Organic Geochemistry Research Group—Determination of Glyphosate, Aminomethylphosphonic Acid, and Glufosinate in Water Using Online Solid-Phase Extraction and High-Performance Liquid Chromatography/Mass Spectrometry

By E.A. Lee, A.P. Strahan, *and* E.M. Thurman

Abstract

An analytical method for the determination of glyphosate, its principal degradation compound, aminomethylphosphonic acid (AMPA), and glufosinate in water with varying matrices has been developed. Four different sample matrices fortified at 0.2 and 2.0 µg/L (micrograms per liter) were analyzed using precolumn derivatization with 9-fluorenylmethylchloroformate (FMOC). After derivatization, cleanup and concentration were accomplished using automated online solid-phase extraction followed by elution with the mobile phase allowing for direct injection into a liquid chromatograph/mass spectrometer (LC/MS). Analytical conditions for MS detection were optimized, and quantitation was carried out using the following representative ions: 390 and 168 for glyphosate; 332, 110, and 136 for AMPA; and 402, 180, and 206 for glufosinate. Matrix effects were minimized by utilizing standard addition for quantification and an isotope-labeled glyphosate (2-^{13}C,^{15}N) as the internal standard. Method detection limits (MDLs) were 0.084 µg/L for glyphosate, 0.078 µg/L for AMPA, and 0.057 µg/L for glufosinate. The method reporting limits (MRLs) were set at 0.1 µg/L for all three compounds. The mean recovery values ranged from 88.0 to 128.7 percent, and relative standard deviation values ranged from 5.6 to 32.6 percent.

INTRODUCTION

Glyphosate [N-(phosphonomethyl)glycine] is a broad-spectrum, nonselective, postemergence herbicide that is used extensively in the United States in various applications for weed and vegetation control. Aminomethylphosphonic acid (AMPA) is a degradation product of glyphosate. Glufosinate [ammonium DL-homoalanin-4-(methyl)phosphinate] is similar to glyphosate in chemical structure and use.

The three compounds are very polar and highly soluble in water. The detection of these compounds requires the use of a derivatization step. Published methods outline the use of precolumn derivatization using 9-fluorenylmethylchloroformate (FMOC) coupled with high-performance liquid chromatography (HPLC) with fluorescence detection (Spark-Holland, 1996) or tandem mass spectrometry (MS/MS) detection (Vreeken and others, 1998). Fluorescence detection has sensitivity but lacks specificity, and the MS/MS method can be subject to matrix variation in derivatization and fragmentation.

Utilization of isotope-labeled (2-^{13}C,^{15}N) glyphosate as an internal standard carried through all steps of a method developed by the U.S. Geological Survey (USGS) Organic Geochemistry Research Group in Lawrence, Kansas, addresses the variations in derivatization and fragmentation for the analysis of glyphosate. Analysis of samples using the standard-addition method, adding a known amount of standard(s) to a matching replicate of each unknown sample further

addresses matrix variations for glyphosate, AMPA, and glufosinate.

The method of analysis described in this report has been assigned the USGS method code “0–2136–01.” This unique code represents the automated method of analysis as it is described in the report and can be used to identify the method.

This report provides a detailed description of the method, including the apparatus, reagents, instrument calibration, and the solid-phase extraction (SPE) procedure required for sample analysis. Method detection limits (MDLs), mean extraction recoveries, and relative standard deviations for the method also are presented.

DETERMINATION OF GLYPHOSATE, AMINOMETHYLPHOSPHONIC ACID, AND GLUFOSINATE IN WATER

Method of Analysis

Scope and Application

The method using HPLC/MS and online SPE is suitable for the determination of low concentrations (in micrograms per liter) of glyphosate, AMPA, and glufosinate in water samples. Associated molecular weights and USGS parameter codes for these compounds and their derivatized compounds are listed in table 1. Because suspended particulate matter is removed by filtration, the method is suitable only for dissolved-phase compounds. The calibration range for the method is equivalent to concentrations from 0.1 to 2.0 µg/L without dilution.

Summary of Method

Water samples are filtered at the collection site using glass-fiber filters with nominal 0.70-µm pore diameter to remove suspended particulate matter. In the laboratory, 10 mL of sample(s) are dispensed into two labeled, 19-mL, screw-capped plastic tubes. The sample in the tube labeled “standard addition” is fortified with 1 µg/L of each compound to be analyzed. Internal standard solutions are added to both tubes, the sample is buffered to pH 9.0 by adding borate buffer, and after mixing, a solution of FMOC is added to all tubes. Derivatization is carried out in the dark in a water bath at 40 °C. After 24 hours, the reaction is stopped and stabilized by adding 2-percent phosphoric acid. All tubes are stored in the dark until analyzed.

A 5.5-mL aliquot of each sample and the matching standard-addition sample are diluted 1:1 with reagent water in autosampler vials, capped, and placed in the tray of the autosampler. The autosampler provides the sample loading for the automated, online SPE system. The SPE cartridge is conditioned with methanol and reagent water. Ten milliliters (10 mL) of the diluted sample are loaded onto the cartridge. After a 500-µL reagent-water rinse, the cartridge is placed into the flow path of the liquid chromatograph (LC) preceding the column. The conditions and gradient of the mobile phase are set to elute the compounds of interest and leave the excess derivatization reagent on the cartridge.

The sample’s compounds are separated by the LC column and detected by the mass spectrometer (MS). Compounds are identified by comparing retention times with the retention times of the standard-addition sample and further by comparision of the selected fragment ions. The concentration of each compound is calculated by determining the ratio of the compound to the internal standard to the ratio of the same compound in the standard-addition sample minus the ratio of the sample. The sample and standard-addition sample are analyzed sequentially, using the same method and instruments.

Table 1. Molecular weights and U.S. Geological Survey parameter codes for glyphosate, aminomethylphosphonic acid, and glufosinate and their FMOC-derivitized compounds

[FMOC, 9-fluorenylmethylchloroformate; --, not applicable]

Compound	Molecular weight (atomic mass units)	Parameter code
Glyphosate	169.1	62722T
Glyphosate-FMOC	391.3	--
Isotope-labeled glyphosate	171.1	--
Isotope-labeled glyphosate-FMOC	393.3	--
Aminomethylphosphonic acid	111.0	62649T
Aminomethylphosphonic acid-FMOC	333.3	--
Glufosinate	181.1	62721T
Glufosinate-FMOC	403.4	--
Cysteic acid	169.2	--
Cysteic acid-FMOC	391.4	--

Apparatus and Instrumentation

- *Analytical balance*—capable of accurately weighing 0.050 0g $\pm$ 0.0001 g.
- *Autopipettes*—10- to 10,000-µL, variable-volume autopipettes with disposable plastic tips (Rainin, Woburn, Massachusetts, or equivalent).
- *Autosampler*—Triathlon, type 900 (Spark-Holland, The Netherlands) equipped with:
  10-mL syringe,
  10-mL sample loop, and
  Type C sample trays (eight each, holding four 20-mm, 10-mL vials).
- *Automated online SPE instrument*—Prospekt, type 795/796–900 (Spark-Holland, The Netherlands).
- *Mechanical vortex mixer.*
- *Water bath.*
- *Analytical column*—Phenomenex Prodigy, 5-µm, 250- x 3-mm C-18 column (Torrance, California).
- *HPLC/MS benchtop system*—Hewlett Packard (Wilmington, Delaware), model 1100 HPLC with autoinjector and MS detector.
  - LC oven conditions: constant 35 ˚C.
  - LC mobile phase: A, 5 mM ammonium acetate in distilled water; B, acetonitrile. Gradient from 5-percent solvent to 17-percent solvent B over 8.5 minutes, 17- to 60-percent solvent B over 10 minutes; 100-percent solvent B for 4 minutes. Flow maintained at 0.5 mL/min.
  - MS detector mode: electrospray in negative-ion mode.
  - Drying gas flow was set at 9 L/min.
  - Nebulizer gas pressure was set at 25 lb/in^2.
  - Fragmentor voltage was set at 70 V.
  - Drying gas temperature was set at 250 ^{o}C.
  - Capillary voltage was set at 3,500 V.
- *Data acquisition system*—computer and printer compatible with the HPLC system.
- *Software*—LC/MSD ChemStation, ver. A.06.03 (Hewlett Packard, Wilmington, Delaware), was used to acquire and store data, for peak integration, and for quantitation of compounds.

Reagents and Consumable Materials

- *Sample bottles*—baked 4-oz amber glass bottles (Boston round) with Teflon-lined lids.
- *Sample filters*—nominal 0.7-µm glass-fiber filters (Gilson, Middleton, Wisconsin, or equivalent).
- *Reagent water*—generated by purification of tapwater through activated charcoal filter and deionization with a high-purity, mixed-bed resin, followed by another activated charcoal filtration, and finally distillation in an autostill (Barnstead, Dubuque, Iowa, or equivalent).
- *Analytical standards*—standards for glyphosate, aminomethylphosphonic acid, glufosinate, cysteic acid, and isotope-labeled glyphosate.
- *SPE cartridges*—Waters Oasis HLB extraction cartridges, Prospekt (10 mm x 2 mm) (Waters, Milford, Massachusetts).
- *Disposable plastic screw-capped tubes*—Fisher 14–959–40B (Fisher Scientific, Pittsburg, Pennsylvania) or equivalent.
- *Plastic rack for tubes.*
- *Solvents*—
  - Acetonitrile, American Chemical Society (ACS) and HPLC grade.
  - Methanol, ACS and HPLC grade.
- *Gas for mass spectrometer*—nitrogen.
- *Ammonium acetate*—ACS grade.
- *Phosphoric acid*—ACS grade.
- *Sodium borate*—ACS grade.
- *9-fluorenylmethylchloroformate*—ACS grade.
- *0.1-mL autosampler vials*—plastic vial with glass-cone insert and cap (Wheaton, Millville, New Jersey).
- *10-mL autosampler vials*—glass vial with Teflon-lined cap (Chromacol, Trumbull, Connecticut).
- *Nebulizer gas*—nitrogen.

Sampling Methods

Sampling methods capable of collecting water samples that accurately represent the water-quality characteristics of the ground water or surface water at a specific time or location are used. Detailed descriptions of sampling methods for obtaining ground-water samples are given in Hardy and others (1989). Similar descriptions of sampling methods used by the USGS for obtaining depth- and width-integrated surface-water samples are given in Edwards and Glysson (1988) and Ward and Harr (1990).

Sample-collection equipment must be free of tubing, gaskets, and other components made of nonfluorinated plastic material that might leach interfering compounds into water samples or absorb the compounds from the water. The water samples from each site are composited in a single container and filtered through a nominal 0.7-µm glass-fiber filter using a peristaltic pump. Filters are preconditioned with about 200 mL of sample prior to filtration of the sample. The filtrate for analysis is collected in baked,

125-mL amber glass bottles with Teflon-lined lids. Samples are chilled immediately and shipped to the laboratory within 3 days of collection. At the laboratory, samples are logged in, assigned identification numbers, and refrigerated at 4 ˚C until derivatized and analyzed.

Standards

- *Primary standard solutions*—Glyphosate, AMPA, and glufosinate were obtained from Chem Service, Inc. (West Chester, Pennsylvania). A solution of 1 mg/mL (corrected for purity) is prepared by accurately weighing, to the nearest 0.0001 g, 50 mg of the pure material into a 50-mL volumetric flask and then diluting with reagent water. The solution is stored at 4 ˚C.
- *Intermediate composite standard*—A 10-µg/mL composite standard is prepared in a plastic container by combining 1 mL of each of the three stock solutions of the compounds with 97 g of reagent water. This composite standard is stored at 4 ˚C. The composite standard is prepared on a monthly basis.
- Standard-addition solution—A 100-µg/L solution is prepared in a plastic container by diluting the intermediate composite standard solution 1:100 with reagent water.
- *Internal-standard solution*—The isotope-labeled glyphosate (2-^{13}C, ^{15}N) is purchased as a 100-µg/mL stock solution from Cambridge Isotope Laboratories, Andover, Massachusetts. A 100-µg/L solution is prepared in a plastic container by adding 20 µL of the stock solution to 20 mL of reagent water.
- *Internal-standard-solution time reference*—50 µg/L of cysteic acid from Sigma, St. Louis, Missouri, in acetonitrile.

Chemical Reagents

- 5 mM ammonium acetate in reagent water (mobile-phase A).
- 2 mM 9-fluorenylmethylchloroformate in acetonitrile.
- 2 percent (volume/volume) phosphoric acid in reagent water.
- 0.1 percent (volume/volume) phosphoric acid in reagent water (rinse solution for autosampler).
- 5 percent (weight/volume) sodium borate in reagent water.
- Acetonitrile (mobile-phase B and rinse solution for autosampler).
- Methanol.
- Reagent water.

Evaluation of High-Performance Liquid Chromatograph/ Mass Spectrometer Performance

Evaluation of Liquid Chromatograph Performance

Background absorbance readings, peak shape, and system pressure are used to evaluate LC performance. Background absorbance readings should remain stable and low and indicate that the LC column has equilibrated with the mobile-phase flow. If peak shape deteriorates, the columns may need to be replaced. If the pressure reading is high, there may be a clog in the mobile-phase flow path, or the column-compartment thermostat may not have reached the required temperature.

Evaluation of Mass Spectrometer Performance

Mass spectrometer performance is evaluated by assessing isotopic ratios and abundance. The MS is tuned in electrospray, negative-ion mode before each HPLC/MS analytical run using the solutions, procedure, and software supplied by the manufacturer.

Calibration

A calibration table is prepared from an analyzed standard using the LC/MSD Chemstation software (Hewlett Packard, Wilmington, Delaware). Manufactures' instructions are followed for using the internal standards as time references and for quantitation.

The relative retention time (*RRTc*) is calculated for each selected compound in the calibration solution or in a sample as follows:

$$RRT_c = \frac{RT_c}{RT_i}, \tag{1}$$

where

RT_c = uncorrected retention time of the selected compound, and

RT_i = uncorrected retention time of the internal standard.

See table 2 for retention times, relative retention times, molecular, and fragment ions.

The expected retention time *(RT)* of the peak of the selected compound needs to be within ± 2 percent of the expected retention time on the basis of the RRT_c

Table 2. Retention times, relative retention times, molecular and fragment ions for FMOC-derivatized compounds of glyphosate, aminomethylphosphonic acid, and glufosinate and internal standards analyzed using method 0–2136–01

[m/z, mass-to-charge ratio; FMOC, 9-fluorenylmethylchloroformate; --, not applicable]

Compound	Retention time (minutes)	Relative retention time (minutes)	Molecular ion (m/z)	Fragment ion 1 (m/z)	Fragment ion 2 (m/z)
Compounds (in order of increasing retention time)					
Glyphosate-FMOC	11.22	1.00	390	168	--
Glufosinate-FMOC	14.35	1.28	402	180	206
Aminomethylphosphonic acid-FMOC	16.31	1.45	332	110	136
Internal standards					
Isotope-labeled glyphosate-FMOC	11.22	1.00	392	170	--
Cysteic acid-FMOC	14.16	1.00	390	168	--

obtained from the internal-standard analysis. The expected retention time is calculated as follows:

$$RT = (RRT_c)(RT_i), \qquad (2)$$

where

RT = expected retention time of the selected compound,

RRT_c = relative retention time of the selected compound, and

RT_i = uncorrected retention time of the internal standard.

Procedure

The samples are derivatized upon arrival in the laboratory, then stored in a refrigerator in the dark until analyzed on the instruments.

- *Sample derivatization*—For each sample, two plastic screw-capped tubes are labeled with the laboratory identification number. The second tube also is labeled standard addition (SA). Ten milliliters (10 mL) of sample are dispensed into each tube. One hundred microliters (100 µL) of the standard-addition solution are added to the SA tube. One hundred microliters (100 µL) of reagent water are added to the first tube. One hundred microliters (100 µL) of the isotope-labeled glyphosate internal standard solution are added to both tubes. One hundred microliters (100 µL) of the cysteic acid internal standard solution are added to both tubes followed by adding 500 µL of 5-percent sodium borate in reagent water. All tubes are mixed by vortexing. One thousand five hundred microliters (1,500 µL) of 2-mM 9-fluorenylmethylchloroformate in acetonitrile are added to all tubes and mixed by inverting at least three times. All tubes are placed in a 40 ^{o}C water bath in the dark for 24 hours, plus or minus 1 hour. The tubes are removed, and 600 µL of 2-percent phosphoric acid in reagent water are added to each tube. Tubes are mixed by inversion at least three times. The derivatized samples then are placed in the refrigerator (in the dark) until analyzed on the instruments. Before analysis, 5.5 mL of each tube is diluted with 5.5 mL of reagent water in the autosampler vial.
- *Sample extraction*—The autosampler, the automated online SPE instrument, and the LC/MS are programmed for the method (see appendices 1 and 2 at the back of this report).
- Each sample and its matching standard-addition sample are loaded into the sample tray of the autosampler. The SPE instrument is loaded with cartridges. The SPE instrument performs one complete cycle of a cartridge before proceeding to the next cartridge (sample). The cartridge is activated with methanol, 2 mL/min for 2 min, and conditioned with reagent water, 2 mL/min for 2 min. Then 10 mL of sample are loaded onto the cartridge from the autosampler at a rate of 2 mL/min. The cartridge is washed with reagent water at the same rate for 15 sec.
- *Sample analysis*—The loaded SPE cartridge is placed in the flow path of the LC/MS prior to the column (using the conditions previously listed). The compounds are eluted using the mobile phase consisting of a gradient beginning with 95-percent mobile-phase A and 5-percent mobile-phase B to 83-percent mobile-phase A and 17-percent mobile-phase B over 8.5 min then changing to 60-percent mobile-phase A and 40-percent mobile-phase B over the next 10 min. A 3.5-min column rinse at 10-percent mobile-phase A and 90-percent mobile-phase B is used. The cartridge remains in the flow path for 9 min.

- *Data acquisition*—The data are acquired using the HP Chemstation software.

Calculation of Results

Qualitative Identification

The LC/MSD Chemstation software (Hewlett Packard, Wilmington, Delaware) is used with the previously prepared calibration table for identification of compounds.

- A compound is not correctly identified unless it has the correct molecular and fragment ions. Additional verification is done by comparing the relative integrated abundance values of the significant ions monitored with the relative integrated abundance values obtained from the standard samples. The relative ratios of the ions need to be within $\pm$20 percent of the relative ratios of those obtained from the standards.
- The retention time (*RT*) of the peak of the selected compound needs to be within $\pm$2 percent of the expected retention time on the basis of the RRT_c obtained from the internal-standard analysis. The expected retention time is calculated using equation 2.

Quantitation

If a selected compound has passed the qualitative identification criteria, the concentration in the sample is calculated as follows:

$$C = \left[\frac{\dfrac{A_c}{A_i}}{\left[\dfrac{A_{csa}}{A_{isa}} - \dfrac{A_c}{A_i}\right]}\right] \bullet SA_c \bullet DF , \qquad (3)$$

where

C = concentration of the selected compound in the sample, in micrograms per liter;

A_c = area of peak of the (molecular or fragment) ion for the selected compound;

A_i = area of peak of the molecular ion for the internal standard;

A_{csa} = area of peak of the (molecular or fragment) for the selected compound in the standard-addition sample;

A_{isa} = area of peak of the molecular ion for the internal standard for the standard-addition sample;

SA_c = concentration of standard addition; and

DF = dilution factor, for samples that have exceeded upper range of method.

Reporting of Results

Glyphosate, AMPA, and glufosinate are reported in concentrations ranging from 0.1 to 2.0 µg/L. If the concentration is greater than 2.0 µg/L, a portion of the original sample is diluted appropriately with reagent water and reanalyzed through the entire procedure.

Method Performance

A reagent-water sample, a ground-water sample collected from a well in Sedgwick County, Kansas, a surface-water sample from the Kisco River below Mt. Kisco, New York, and a surface-water sample from the spillway below Clinton Lake in Kansas were used to test the performance of method 0–2136–01. All samples were filtered through a nominal 0.7-µm glass-fiber filter and stored at 4 ^{o}C.

Samples of each matrix were spiked with glyphosate, AMPA, and glufosinate to concentrations of 0.2 and 2.0 µg/L and analyzed on different days during August 2001. In addition, unspiked samples of each matrix were analyzed. Comparisions of the different matrices and concentrations included bias from day-to-day variations. Method recoveries from the analyses are included in tables 3–6.

Corrections for Background Concentrations

The reagent-water sample, ground-water sample, and surface-water sample from the Kisco River did not require correction for background concentrations of glyphosate, AMPA, and glufosinate. The surface-water sample from Clinton Lake contained glyphosate at 0.31 µg/L (table 6) but did not contain AMPA or glufosinate. The data from this water sample were corrected for the background concentration of glyphosate.

Method Detection Limits

A method detection limit (MDL) is defined as the minimum concentration of a substance that can be identified, measured, and reported with a 99-percent confidence that the compound concentration is greater than zero. MDLs were determined according to pro-

Table 3. Mean recoveries and standard deviations for glyphosate, aminomethylphosphonic acid, and glufosinate in reagent-water samples analyzed using method 0–2136–01

[µg/L, microgram per liter]

Compound	Eight samplesss piked at 0.2 µg/L				Eight samples spiked at 2.0 µg/L			
	Mean recovery		Standard deviation	Relative standard deviation	Mean recovery		Standard deviation	Relative standard deviation
	(µg/L)	(percent)	(µg/L)	(percent)	(µg/L)	(percent)	(µg/L)	(percent)
Glyphosate	0.199	100	0.028	14	2.14	107	0.19	9
Aminomethylphosphonic acid	.224	112	.026	12	2.41	120	.38	16
Glufosinate	.220	110	.019	9	2.57	129	.18	7
Average	**.214**	**107**	**.024**	**11**	**2.37**	**119**	**.25**	**11**

Table 4. Mean recoveries and standard deviations for glyphosate, aminomethylphosphonic acid, and glufosinate in ground-water samples analyzed using method 0–2136–01

[µg/L, microgram per liter]

Compound	Eight samples spiked at 0.2 (µg/L)				Eight samples spiked at 2.0 (µg/L)			
	Mean recovery		Standard deviation	Relative standard deviation	Mean recovery		Standard deviation	Relative standard deviation
	(µg/L)	(percent)	(µg/L)	(percent)	(µg/L)	(percent)	(µg/L)	(percent)
Glyphosate	0.194	97	0.040	21	2.04	102	0.46	23
Aminomethylphosphonic acid	.228	114	.054	24	2.24	112	.62	28
Glufosinate	.220	110	.037	17	2.53	126	.45	18
Average	**.214**	**107**	**.044**	**20**	**2.27**	**113**	**.51**	**23**

Table 5. Mean recoveries and standard deviations for glyphosate, aminomethylphosphonic acid, and glufosinate in surface-water samples from Kisco River analyzed using method 0–2136–01

[µg/L, microgram per liter]

Compound	Eight samples spiked at 0.2 (µg/L)				Eight samples spiked at 2.0 (µg/L)			
	Mean recovery		Standard deviation	Relative standard deviation	Mean recovery		Standard deviation	Relative standard deviation
	(µg/L)	(percent)	(µg/L)	(percent)	(µg/L)	(percent)	(µg/L)	(percent)
Glyphosate	0.188	94	0.040	21	1.93	96	0.49	26
Aminomethylphosphonic acid	.233	117	.043	18	2.23	111	.56	25
Glufosinate	.235	118	.044	19	2.52	126	.82	33
Average	**.219**	**109**	**.042**	**19**	**2.22**	**111**	**.62**	**28**

Table 6. Mean recoveries and standard deviations for glyphosate, aminomethylphosphonic acid, and glufosinate in surface-water samples from Clinton Lake analyzed using method 0–2136–01

[µg/L, microgram per liter]

Compound	Eight samples spiked at 0.2 (µg/L)				Eight samples spiked at 2.0 (µg/L)			
	Mean recovery		Standard deviation	Relative standard deviation	Mean recovery		Standard deviation	Relative standard deviation
	(µg/L)	(percent)	(µg/L)	(percent)	(µg/L)	(percent)	(µg/L)	(percent)
Glyphosate[1]	0.176	88	0.044	25	2.32	116	0.43	19
Aminomethylphosphonic acid	.204	102	.038	19	2.02	101	.29	14
Glufosinate	.208	104	.012	6	2.32	116	.37	16
Average	**.196**	**98**	**.031**	**16**	**2.22**	**111**	**.36**	**16**

[1]Water from Clinton Lake contained 0.31 µg/L of glyphosate; above values are corrected for background concentrations.

cedures outlined by the U.S. Environmental Protection Agency (1992). Eight replicate samples of buffered reagent water spiked with 0.20 µg/L of each of the compounds were analyzed to determine MDLs (table 7). Each sample was analyzed on different days during August 2001 so that day-to-day variation is included in the results.

The MDL was calculated using the following equation:

$$MDL = (S)(t_{(n-1,\,1-\alpha\,=\,0.99)})\,, \qquad (4)$$

where

S = standard deviation of replicate analysis, in micrograms per liter, at the spiked concentration;

$t_{(n-1,\ 1-\alpha = 0.99)}$ = Student's *t*-value for the 99-percent confidence level with *n*-1 degrees of freedom (U.S. Environmental Protection Agency, 1992); and

n = number of replicate analyses.

The estimated mean MDL was 0.084 µg/L for glyphosate, 0.078 µg/L for AMPA, and 0.057 µg/L for glufosinate (table 7). According to the U.S. Environmental Protection Agency (1992) procedure, the spiked concentrations should be no more than five times the estimated MDL. The spiked concentrations were within five times the MDL.

Mean Recovery

Mean recoveries in reagent-water, ground-water, and surface-water samples were determined by comparing the mean analyzed concentration (see "Quantitation" section) from the eight replicate samples to the spiked concentration. Mean recoveries were highest overall in reagent-water samples at the 2.0-µg/L level and lowest overall in the Clinton Lake water at the 0.2-µg/L level. Relative standard deviations of the recoveries ranged from 5.8 to 32.6 percent.

Table 7. Mean concentrations, standard deviations, and method detection limits for eight determinations of glyphosate, aminomethylphosphonic acid, and glufosinate spiked at 0.20 microgram per liter in eight samples of reagent water analyzed using method 0–2136–01

[µg/L, microgram per liter]

Compound	Eight samples spiked at 0.2 µg/L: Mean concentrations (µg/L)	Standard deviation (µg/L)	Method detection limit (µg/L)
Glyphosate	0.199	0.028	0.084
Aminomethylphosphonic acid	.224	.026	.078
Glufosinate	.220	.019	.057
		Minimum	**.057**
		Maximum	**.084**

DISCUSSION

An HPLC method utilizing online SPE and fluorescence detection was reported by Spark-Holland (1996) as being quite sensitive but lacking the confirmation available using mass spectrometry. Also, the mobile phase used was not readily compatible with most mass spectrometers currently (2001) in use. The use of disposable SPE cartridges, as outlined by Spark-Holland (1996), improves LC column life and reduces the possiblity of carryover.

An HPLC/MS/MS method utilizing online SPE was reported by Vreeken and others (1998). This HPLC/MS/MS method used the same column repeatedly for cleanup and lacked the accuracy afforded by the use of internal standards. Both of the HPLC and HPLC/MS/MS methods used precolumn derivatization with FMOC.

An GC/MS/MS method utilizng ion-exchange chromatography followed by derivatization was reported by Royer and others (2000). The GC/MS/MS method required elaborate preparation of columns and an elaborate derivatization procedure.

The incorporation of an isotope-labeled (2-^{13}C, ^{15}N) glyphosate as an internal standard carried through the derivatization, extraction, separation, and detection steps enhanced the reproducibility and accuracy of the online SPE and HPLC/MS method described in this report. The use of standard addition for quantitation overcomes the variation in derivatization and fragmentation observed from analyzing compounds with different matrices. The use of the more readily available single quadrapole mass spectrometer allowed for simpler and less-expense operation.

Figure 1 shows a total ion chromatogram of a FMOC-derivatized, 2.0-µg/L spiked surface-water sample from Clinton Lake. Figure 2 shows the chromatograms of the FMOC-derivatized ions of each compound. Baselines are relatively clean, and separations are adequate for quantitation. The cysteic acid internal standard, which was used before the labeled glyphosate became available, has been left in the method as a retention-time reference.

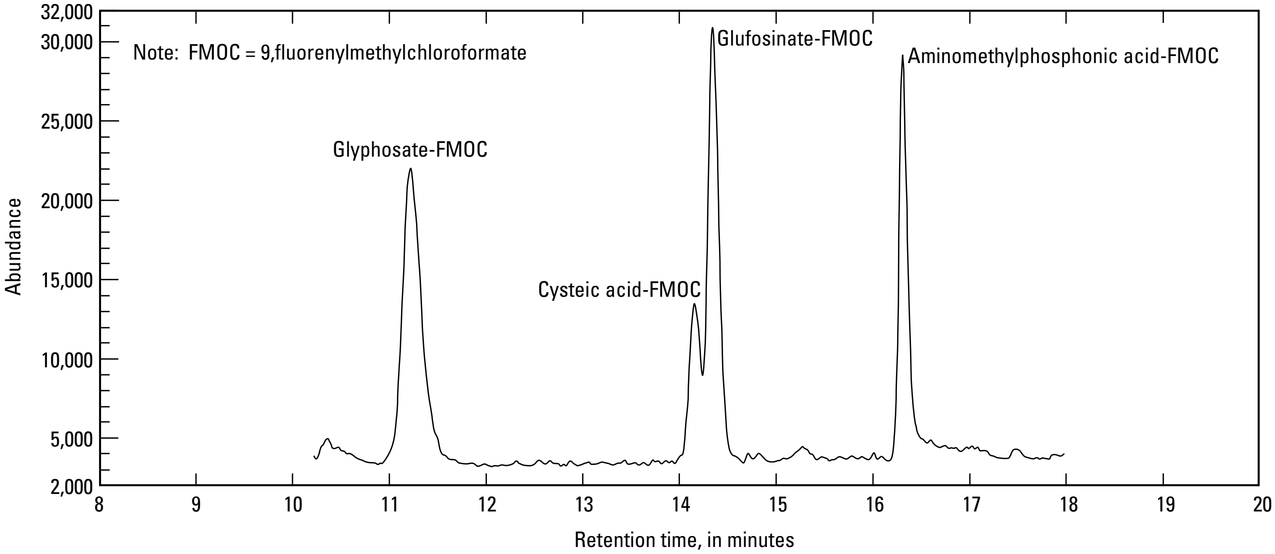

Figure 1. Total ion chromatogram of a FMOC-derivitized 2.0-microgram-per-liter spiked surface-water sample from Clinton Lake analyzed using method 0–2136–01.

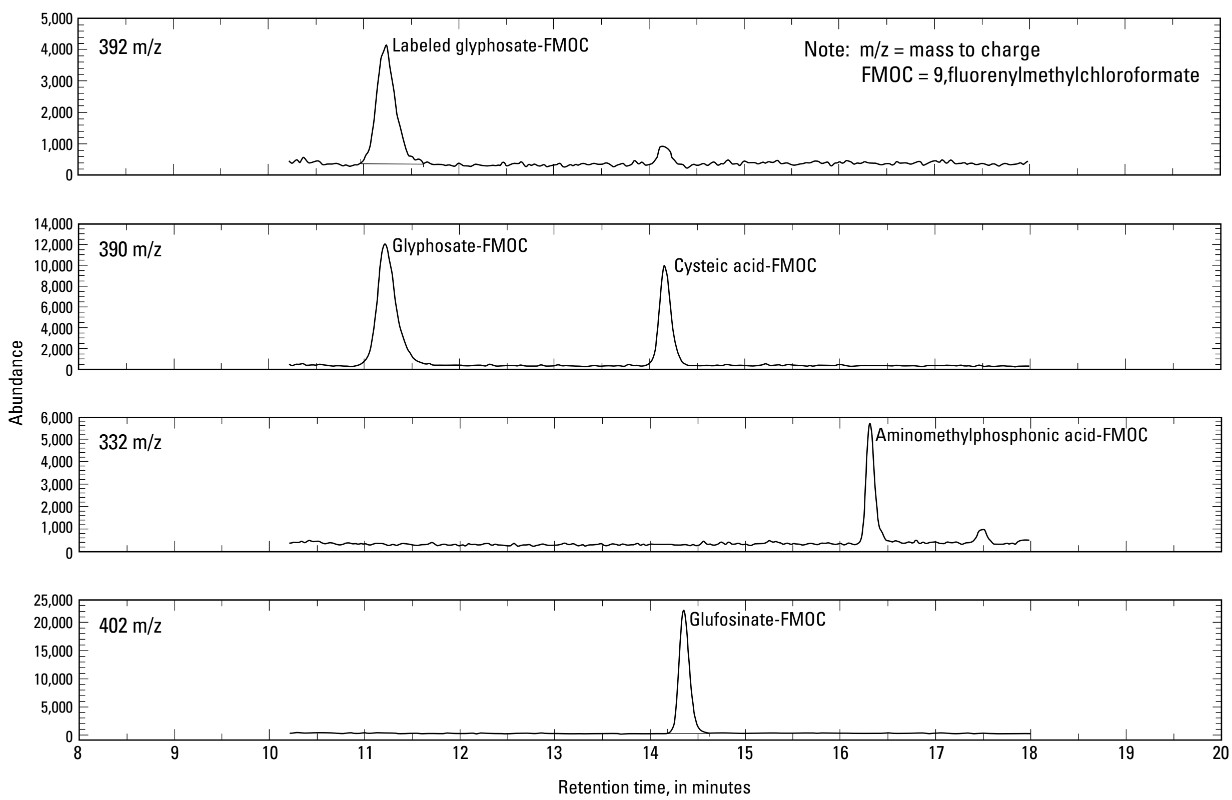

Figure 2. Selected ion chromatograms of a FMOC-derivitized 2.0-microgram-per-liter spiked surface-water sample from Clinton Lake for molecular-FMOC ions 392, 390, 332, and 402 analyzed using method 0–2136–01.

CONCLUSIONS

The method described in this report provides for routine analysis of glyphosate, AMPA, and glufosinate in environmental water samples. Derivatization with FMOC, online SPE, and HPLC/MS are shown to be a reliable and sensitive method for low concentrations.

Good precision and accuracy for the analysis of glyphosate, AMPA, and glufosinate were demonstrated for reagent water, ground water, and surface water. Method detection limits were 0.084 μg/L for glyphosate, 0.078 μg/L for AMPA, and 0.057 μg/L for glufosinate. The mean recoveries of water samples spiked at 0.2 and 2.0 μg/L ranged from 88.0 to 128.7 percent with relative standard deviations ranging from 5.8 to 32.6 percent.

Information about the fate and transport of glyphosate, its degradation compound AMPA, and glufosinate in water can be acquired from the analysis of ground water and surface water. This method also can be used for water-quality determinations.

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APPENDICES

Appendix 1. Programmed steps for autosampler

Glyphosate method 0–2136–01

Basic system parameters:
Wash solution: 0.1-percent phosphoric acid in reagent water.
User program (only program available using 10-mL vials).
Loads 10 mL of sample on cartridge in online automated solid-phase extraction (SPE) instrument.
10-mL syringe; 10-mL sample loop; 15-mL buffer loop
(sample loop calibrated at 9.9 mL).
Type C: four vials by eight sample tray segments.

Step	Action	Function	Notes
1	Wait	5 secJ	
2	Aux port 3	OFF	Unfreezes online SPE instrument.J
3	Compressor	ONJ	
4	Syringe valve	NeedleJ	
5	Injection valve	LoadJ	
6	Aspirate	10,000-µL sample-speed 5-height 4 mm	Draws sample into sample loop.J
7V	Wait for input 2	Low	Waits for signal from online SPE instrument that cartridge preparation is done.
8	Injection valve	Inject	Puts sample loop into cartridge path.
9	Dispense	2,000-µL waste-speed 4	Empties excess sample from buffer loop to waste.
10	Syringe valve	Wash	
11	Aspirate	2,000-µL wash-speed 5	Refills syringe with wash solution.
12	Syringe valve	Needle	
13	Wait	10 sec	
14	Wait for input 2	Low	Waits for signal from online SPE instrument that cartridge preparation is done.
15	Injection valve	Load	Removes sample from loop from cartridge path.
16	Wait	10 sec	
17	Dispense	10,000-µL waste-speed 5	Dispenses syringe contents through buffer loop and sample loop for cleaning.
18	Syringe valve	Wash	
19	Load syringe	Volume 10,000-µL-speed 9	Refills syringe with wash solution.
20	Syringe valve	Needle	
21	Dispense	10,000-µL waste-speed 5	Dispenses syringe contents through buffer loop and sample loop for cleaning.
22	Needle wash	300 µL	Washes needle.
23	Compressor	OFF	
24	Wait for input 2	Low	Waits for signal from online SPE instrument that cartridge preparation is done.
25	Wait	3 sec	
26	Aux port 3	ON	Freezes online SPE instrument.
27	Wait for input 1	Low	Waits for signal from LC/MS Chemstation generated by the injection.
28	Wait	5 sec	
29	END		Returns to step 1 until all vials in series are done.

Appendix 2. Programmed steps for automated online solid-phase extractor

Glyphosate method 0–2136–01

Sample preparation program

Solvent 1 Methanol
Solvent 2 Acetonitrile
Solvent 3 Distilled, deionized water

SP programV # 12:
Elutes previously loaded cartridge to HPLC column.
Prepares the next cartridge.
Loads 10 mL from sample loop of autosampler onto the SPE cartridge.
Rinses line from autosampler to online SPE instrument.

Step	Time (minutes)	Action
1	0.00	Valve 1 purge
2	.05	Valve 1 elute
3	9.05	Valve 1 purge
4	9.15	Change cartridge
5	9.15	Solvent 1
6	9.15	2.0 mL/min
7	11.16	Solvent 3
8	13.16	Aux 2 ON
9	13.16	Aux 2 OFF
10	13.17	2.0 mL/min
11	18.30	0.0 mL/min
12	18.35	Aux 2 ON
13	18.36	Aux 2 OFF
14	18.40	Valve 2 * * *
15	18.41	Solvent 2
16	18.42	2.0 mL/min
17	19.41	Solvent 1
18	20.11	0.0 mL/min
19	20.12	Valve 2 - - -
20	23.43	Aux 2 ON
21	23.44	Aux 2 OFF
22	23.49	End of program